

PLANETARY PROTECTION TECHNOLOGIES AT THE JET PROPULSION LABORATORY¹

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ABSTRACT

This paper presents a summary of the planetary protection technology development efforts currently being conducted at the Jet Propulsion Laboratory (JPL). This is a multidisciplinary effort involving JPL and NASA expertise in microbiology, material science, and analytical chemistry, in conjunction with outside, industrial and academic collaborators. The technologies under development include: bioburden reduction technologies (cleaning and sterilization); biological cleanliness validation technologies, archiving technologies, and hardware cleanliness maintenance technologies.

INTRODUCTION

What is Planetary Protection?

The National Aeronautics and Space Administration (NASA) maintains a policy of planetary protection to limit the contamination of extraterrestrial bodies by terrestrial microorganisms and organic compounds during spaceflight missions. This policy and the planetary protection procedures developed to implement this policy are consistent with the biological contamination control objectives of the Committee on Space Research (COSPAR) of the International Council of Scientific Unions and of Article IX of the Treaty on Principles Governing the Activities of States in the Exploration and Use of Outer Space, January 27, 1967, TIAS 6347 (entered into force October 10, 1967).

NASA's policy on planetary protection is presented in the NASA Policy Directive, NPD 8020.7E⁽¹⁾, "Biological Contamination Control for Outbound and Inbound Planetary Spacecraft." The NASA Procedures and Guidelines document, NPG 8020.12B⁽²⁾ entitled "Planetary Protection Provisions for Robotic Extraterrestrial Missions," sets forth

requirements applicable to robotic planetary flight programs in accordance with NPD 8020.7E. The requirements are specifically directed to the control of:

- (1) forward contamination, i.e. terrestrial microbial contamination associated with robotic space vehicles intended to land, orbit, flyby, or otherwise be in the vicinity of extraterrestrial solar system bodies, and
- (2) back contamination of the Earth and Moon by extraterrestrial solar system material collected and returned by such missions.

Minimizing the risk of forward contamination is based on the imperative to preserve the scientific integrity of the planetary body under study, and by the imperative to preserve and protect any indigenous organisms from possible harm by introduced terrestrial life. The requirements in NPG 8020.12B apply to all planetary flight activities, including solar system exploration missions to the major planets as well as missions to planet satellites and other solar system objects that may be of scientific interest, with the following exceptions:

- (1) terrestrial (Earth-orbiting) missions;
- (2) lunar missions;
- (3) human missions, except for Shuttle-launched, but otherwise robotic, planetary missions.

Planetary Protection Categories and Requirements

NASA's current planetary protection requirements are based on our understanding of the biological potential of Mars from the findings of the Viking missions of the mid-1970's and on a policy adopted at COSPAR's 25th General Assembly.⁷ This policy was subsequently refined in a 1992 National Research Council's (NRC) Space Studies Board report, "Biological Contamination of Mars: Issues and Recommendations,"^{6,8} The NRC's 1992 report recommending the distinction between Mars landers with and without in situ life experiments was later codified and adopted by COSPAR.^{11,12}

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The planetary protection requirements are determined by both the mission type (e.g., flyby, orbiter, or lander) and the interest of the extraterrestrial solar system body to the chemical evolution and/or origin of life. Each planetary mission falls into one of five categories as summarized in Table 1, with each category having different planetary protection requirements as dictated in NPG 8020.12B. Categorization of a mission is established between NASA's Planetary Protection Officer (PPO) and the Flight Program Manager. Planetary protection requirements are also intended to apply to the flight of NASA instruments and/or experiments manifested on non-NASA spacecraft.

Table 1. Planetary Protection Mission Categories

	Planet Priorities	Mission Type	Mission Category
A	Not of direct interest for understanding the process of chemical evolution. No protection of such planets is warranted and no requirements are imposed.	Any	I
B	Of significant interest relative to the process of chemical evolution but only a remote chance that contamination by spacecraft could jeopardize future exploration.	Any	II
C	Of significant interest relative to the process of chemical evolution and/or the origin of life or for which scientific opinion provides a significant chance of contamination which could jeopardize a future biological experiment.	Flyby, Orbiter	III
		Lander, Probe	IV
All	Any solar system body	Earth- Return	V

Detailed requirements for each mission Category can be found in NPG 8020.12B and in JPL D-18699, "Condensed Planetary Protection Design Guide." Briefly, for U.S. missions, certification as Category I relieves a project of all further PP requirements, including documentation. Missions to bodies typically not requiring planetary protection provisions include missions to and from the Earth's Moon, and missions to the Sun and Mercury. For U.S. missions with Category II and higher certification, planetary protection requirements include any or all of the following:

- Planetary Protection Plan that must detail the planned approach to compliance with the implementation requirements,
- Pre-Launch Planetary Protection Report that must document the degree to which all requirements have been met,
- Post-Launch Planetary Protection Report that must update the Pre-Launch Planetary Protection Report,
- End-of-Mission Report which provides a complete report of compliance, the final actual disposition of launched hardware,
- impact avoidance, orbital lifetime limits, and contamination control requirements,
- bioburden reduction procedures of cleaning and/or sterilization, concomitant bioassays to establish the bioburden levels, and protection of assembled spacecraft and all modules that have been bioassayed against recontamination,
- inventory, document and possible archiving of spaceflight hardware organic materials and the mass used.
- for landed missions, locations of landings and impact points of major components of space vehicles on the planet surface

NPG 8020.12B makes a clear distinction between the requirements for missions that carry life detection experiments (Category IVB) and those that do not (Category IVA). Category IVA missions, comprising lander and probes without life-detection experiments, must meet a bioburden limit specified for exposed surfaces. Category IVB, lander and probes with life-detection experiments, requires a more stringent bioburden limit and a complete system sterilization. A distinction is also drawn between unrestricted and restricted Earth-return missions. For restricted Earth-return missions, requirements may affect all phases of the mission, namely the outbound leg; sample acquisition, transfer and storage; sealing of the sample container; monitoring of the sample; return phase of the mission; Earth entry phase; and sample receiving laboratory.

PLANETRY PROTECTION AT JPL

JPL is actively developing new technologies to enable future in situ life detection and sample return mission to meet their planetary protection requirements. These development efforts are being conducted under the auspices of the Mars Program and are designed to both enable near term missions and anticipate changes in future planetary protection requirements.

Planetary protection technologies are divided as:

- (1) Bioburden reduction technologies
 - i. cleaning - the removal of biological contamination, including particles, viable/nonviable organisms and residues, from hardware surfaces and
 - ii. sterilization - the elimination of viable organisms on hardware surfaces;
- (2) Validation - determining biological decontamination effectiveness by
 - i. quantifying the amount of any remaining biological contamination and
 - ii. identifying the nature of any remaining biological contamination;
- (3) Archiving - recording the biological history of hardware materials, assembly area, spacecraft, and launch site against future planetary protection and science requirements; and
- (4) Maintenance - identifying/minimizing/removing any cross contamination or recontamination during assembly, functional hardware test, transit, rework at remote facilities, any landing, and any Earth return. This includes methods of modeling cross contamination.

Bioburden Reduction and Validation Technologies

Two approaches to bioburden reduction can be taken: cleaning and sterilization. Indeed, a twofold approach was employed for Viking where bioburden reduction was performed by cleaning, followed by heat sterilization. The Viking landers were carefully cleaned (using hot He?) and assembled in Class 100,000 clean rooms. Once sealed inside their bioshields, their bioburden were further reduced by dry heating sterilization conducted at a humidity of 1.3 mg/liter such that at the coldest point a temperature of 111.7 °C was maintained for 30 hours.

For Viking, and subsequent missions to Mars, hardware bioburden was defined by the number of cultivable spores/m² present on the spacecraft. ⁽²⁵⁾ The planning of sample return missions to Mars has recently to an extension of the definition of bioburden to include biological contamination arising from:

- (1) viable prokaryotic organisms, i.e., intact microbes and spores possibly capable of growth;
- (2) nonviable organisms which contaminate but do not have the propensity to grow, including any intact, nonviable microbial and spore "ghosts," and any residual associated biosignature molecules;
- (3) eukaryotic species, such as human cells, their desquamata, insect frass, and yeast, algae, fungi and protozoa and their associated biosignature molecules;
- (4) particles, in so far as they are able to harbor any of the above contaminants.

Cleaning Technologies

Currently, flight hardware undergoes routine gross cleaning to remove major contaminants, followed by fine or precision cleaning, as needed. JPL follows the specification outlined in documents FS504574RevC⁽¹⁸⁾ and FS505146D.⁽¹⁷⁾ FS504574 Rev.C propulsion cleaning calls for the use of isopropyl alcohol (IPA) for the parts exposed to fuel, and freon for parts exposed to oxidizers. Precision cleaning is not covered as a separate item in this procedure. However, the MIL-STD-1246C⁽²⁰⁾ calls out this method as one which is used to achieve a level of product cleanliness that is greater than the level normally detected by visual means. Surfaces of assembled flight hardware are routinely cleaned using isopropyl alcohol wipes.

Sterilization Technologies

Dry heat sterilization is a very effective way of achieving sterilization and is currently the only accepted method for flight hardware bioburden reduction using sterilization (ref). The effectiveness of this technique depends on:

- (1) the time-temperature profile,
- (2) water activity in the system (organisms and environment),
- (3) whether the system is closed or open,
- (4) heat resistance of the microorganisms,
- (5) physical properties of the contaminated materials, and
- (6) characteristics of the chamber atmosphere ⁽²⁴⁾.

Open and closed systems represent the two extremes in water transport to or from the microorganisms during heating. In open systems, the microbes are in intimate and continuous contact with the surrounding atmosphere while they undergo dry heat sterilization; in closed systems, the microbes are located so that they are completely surrounded by a solid material impervious to water vapor transmission.

In the temperature range of 100 to 125°C, inactivation is strongly dependent upon relative humidity (RH), with lethality above 50% RH and below 0.2% RH much greater than it is in the intermediate range. Wet sterilization of space flight hardware is not acceptable

because it degrades reliability of delicate components; thus, the dry end of the RH spectrum is used for U.S. flight projects.

The maximum temperature of the sterilization cycle is determined by the heat sensitivity of the component to be sterilized. A minimum of 111.7°C has been quoted to be the minimum temperature required for the heating plateau⁽²⁴⁾; however, times at temperatures above 100 °C can be used for computing the lethality attributable to the heating and cool-down of the cycle. If a lower temperature is used, the time to achieve the same level of sterility as at the higher temperature is increased. This detailed procedure is discussed in Appendix V of Planetary Protection Guide Book (JPL D-18635).

In the mid-1970's, heat sterilization was considered to be most effective approach to killing known microorganisms and as such the Viking landers were specifically designed to undergo a dry heat sterilization process. Our current knowledge of the existence of heat tolerant, extremeophile microorganisms has lead to the need for more advanced – and effective – sterilization procedures.

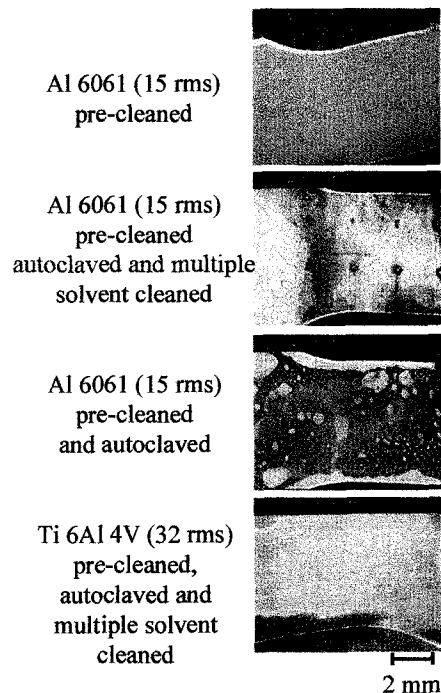
One such procedure makes use of hydrogen peroxide vapor with or without a gas plasma. In this approach, a 30% to 59% solution of hydrogen peroxide is vaporized into a chamber at a temperature of 25 to 60°C and pressure of 6 to 10 torr. The chamber temperature and pressure are set to ensure that there is no condensation of either water or hydrogen peroxide vapor. In the case of plasma sterilization, an electromagnetic field is created in the chamber to aid in sterilization efficacy⁽⁵⁾.

Biological Cleanliness Study

As part of our planetary protection research and development effort at JPL we have undertaken a detailed study of the biological cleaning effectiveness of a number of accepted cleaning procedures. For the study, biological cleaning effectiveness is defined in terms of the ability to remove viable bacterial spores. In the study, coupons of two flight hardware materials deemed most likely to be used in the construction of sample handling hardware devices, Al 6061 (mill finish 15 rms and 2 rms) and Ti 6Al-4V (mill finish 32 rms), were precleaned, autoclaved, and then inoculated with 10³ viable (10⁴ total) *Bacillus subtilis* spores. The inoculated coupons were then cleaned using: a standard JPL cleanroom polyester wipe wetted with 70% IPA; a standard wipe wetted with ultrapure water (UPW); detergent cleaning followed by a UPW water rinse; a standard, multiple solvent, flight hardware cleaning procedures for Al 6061 and Ti 6Al-4V (per FS505146 Rev.C); a

commercial, semi-aqueous cleaning process; and a commercial, oxygen plasma cleaning method. Why chosen? Materials compatibility concerns and measurements.

Cleaning effectiveness was then measured using NASA standard agar plate assay (NPG: 5340.1C), diffuse reflectance Fourier transform infrared spectroscopy (DRIFT/FTIR, as per MIL-STD 1246C), and the more advanced biological validation methods of limulus ameobocyte lysate (LAL), environmental scanning electron microscopy (ESEM), and electron diffraction spectroscopy (EDS). LAL is a very sensitive, highly specific assay that measures the presence of Gram negative bacteria.¹⁹ The LAL assay is a commercially available assay that takes advantage of the unique immune response initiated by *Limulus polyphemus* (horseshoe crab) when a Gram negative bacteria enters its system. Since LAL detects only Gram negative bacteria, it was used to trace contamination during the study. ESEM is used not only to interrogate the surface morphology of the materials after cleaning, but combined with EDS it can detect and confirm the presence or absence of biological remnants on the surface.



Results of the study show that:

- all cleaning methods studied clean spores to some degree, except the plasma cleaning procedure which proved ineffective at removing spores inoculated at this level;
- Ti 6Al-4V (mill finish 32) can be "cleaned-to-sterility" using multiple-solvent cleaning method

(JPL method FS505146 Rev.C) with *no* spore remnants seen, where sterility is defined as the absence of growth in tryptic soy broth;

- unpolished Al 6061 *cannot* be cleaned to sterility with any of the cleaning methods studied in the matrix, including the procedure spelled out for Al 6061 in FS505146 Rev.C;
 - however, when the FS505146 Rev.C procedure for Al 6061 is *modified* to include a nitric acid passivation step per the procedure for Ti 4Al-6V, Al 6061 with an 15 rms and 2 rms can be "cleaned-to-sterility," where sterility is defined above;
 - requisite autoclaving step leads to extensive oxidation of surface properties of hardware materials, thus results represent "worst case" cleaning scenario;
- Pictures of cleaned Al and Ti surfaces?

We are currently undertaking a reduced cleaning matrix involving two additional hardware materials – Chemfilmed Al 7075 and Huges M1 painted Al 7075. Coupons of these materials will be inoculated with *B. subtilis* spores and cleaned using less stringent cleaning methods, including: 70% IPA wipe, detergent cleaning followed by a UPW water rinse, and cleaning with TBD antibacterial agents. Results of this study are forthcoming.

Sterilization Materials Compatibility Study

Current planetary protection methods for various missions include wiping or cleaning hardware with alcohol and then bioassaying the surfaces. For hard to clean surfaces, a dry heat process is used to reduce the microbial burden. The disadvantages of the dry heat process are the high temperatures involved (105 to 125°C) and long processing time (5 to 50 hours). The hydrogen peroxide plasma sterilization process involves low process temperatures (50 to 55°C) and short process time (approximately one hour). Some common spacecraft materials tested to-date include metals, plastics, adhesives, tapes, lubricants, circuit board coatings, paints, thermal blankets, and miscellaneous hardware components. A list of materials and their properties before and after exposure to hydrogen peroxide will be presented. The results show good compatibility with spacecraft hardware tested and that the hydrogen peroxide plasma process shows great promise as an alternative spacecraft hardware sterilization technique. Representatives of Gram-positive and Gram-negative bacterial species procured from American Type Culture Collection were artificially seeded onto aluminum coupons and subjected to hydrogen peroxide sterilization.

Conventional culture techniques revealed that all the tested bacterial species were killed by 2 to 4-injections of hydrogen peroxide treatments. The hydrogen peroxide-treated coupons analyzed *in situ* by environmental scanning electron microscopy revealed remnants for all microbes tested. The microbial structure of the Gram-positives was intact when compared to the Gram-negatives. However, these samples did not yield any PCR-amplifiable 16S rDNA fragments. Likewise, microbial species isolated from Jet Propulsion Laboratory Spacecraft Assembly Facility were subjected to various doses of hydrogen peroxide treatment to determine their sensitivity.

Validation Technologies

The current, standard NASA method of hardware cleaning validation for planetary protection involves a measure of the number of cultivable (capable of growth under the conditions specified in the procedure) microorganisms present.⁽²⁵⁾ There are specified methods for aerobic spores (sporeformers), aerobic spore and non-sporeformers total, anaerobic spores, and anaerobic spores and non-sporeformers total. The technique for aerobic spores (as required for Category IVA missions, for example) involves sampling a 26 cm² portion of the hardware using a sterilized cotton swab wetted with sterile, distilled water. The swab is then vortexed and sonicated in distilled water to remove the microbes and other bioburden species and the solution is then heat-shocked at 80°C to kill all cells, except the spores which survive this heat treatment. The spores containing solution is then transferred onto an agar gel, allowed to culture for three days at 32°C and then the cultures are counted. For aerobic spores and non-sporeformers total, the heat shock step is omitted. For anaerobes, the germination and growth is conducted under anaerobic conditions in Brewer jars.

It is now known that less than 10% of known microbes form spores, and of these, less than 1% of are amenable to culturing. Thus, the simple measure of culturable spores underestimate the population of microbes – and thus the hardware bioburden - by a factor of about 1000. In addition, the standard NASA method does not measure any of the nonviable organisms (microbial "ghosts"), biosignature molecules, or eukaryotic biological contamination which are now a part of hardware cleanliness validation. This, in conjunction with the extension of the definition of cleanliness noted above has led to the need for more comprehensive and sensitive methods for validating hardware cleaning to meet planetary protection requirements.

Our advanced cleaning validation technologies development efforts at JPL have focused on the following methods:

- (1) whole cell counting using epifluorescence microscopy;
- (2) limulus amebocyte lysate (LAL) detection of gram negative bacteria;
- (3) DNA analysis using polymerase chain reaction (PCR);

Archiving

In ongoing investigations to map and archive the microbial footprints in various components of spacecraft and its accessories, we have examined the microbial populations of the Spacecraft Assembly Facility (SAF). We have exposed witness plates that are made up of spacecraft materials and or painted

accumulated the highest number of particles, hence attracted more cultivable microbes and spore-formers. The microbiological examination revealed that the SAF High Bay-1 harbors mainly Gram-positive microbes and mostly spore-forming *Bacillus* species. Most of the isolated microbes were heat resistant to 80°C for 15 min., grew well at 60°C, and in the presence of 10% NaCl concentration. Some of the isolates were resistant to H₂O₂ treatment that kills majority of the microbes. Based on the morphology, and physiology, 28 isolates were chosen for further study. The phylogenetic relationships among these heat-tolerant microbes were examined using a battery of morphological, physiological, and molecular characterizations. Among 28 strains tested for their phenotypic characterization, only 8 strains were identified as to species. This may be due to the fact that the commercially available identification kit was not designed to identify unknown bacterial species that are found in the extreme environment. The fatty acid methyl ester profiles of 80% of the tested isolates matched with known bacterial species. Sequence analysis of nearly complete sequences of 16S ribosomal RNA revealed that most of the microbes in SAF are *Bacillus licheniformis*. By 16S rRNA analysis, the isolates fell into seven clades *Bacillus licheniformis*, *B. pumilus*, *B. cereus*, *B. circulans*, *Staphylococcus epidermidis*, and representatives of the genera *Planococcus* and *Micrococcus*. In addition to the *Bacillus* species (22 isolates), the isolation of human-associated microbes such as *S. epidermidis* indicates secondary contamination by human activity in SAF. DNA-DNA hybridization studies were carried out between the SAF strains and species procured from various culture collections.

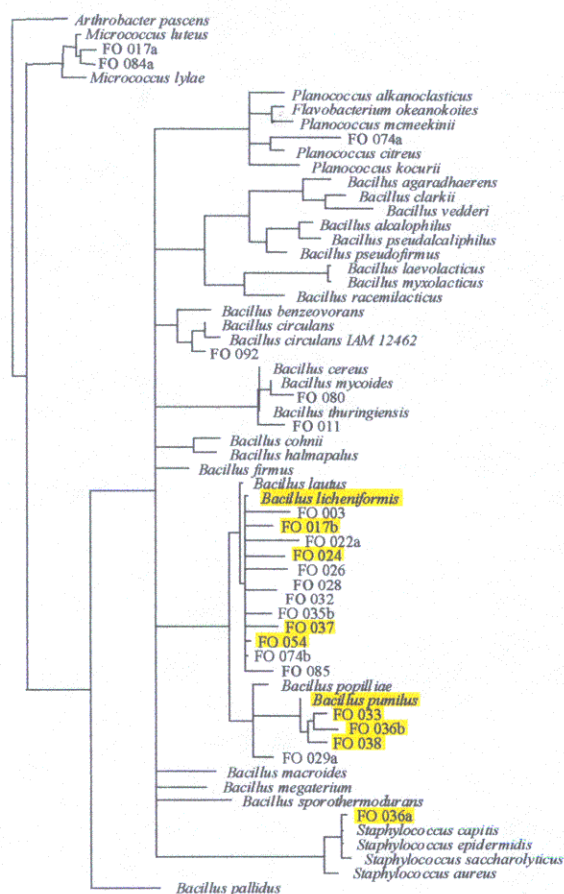
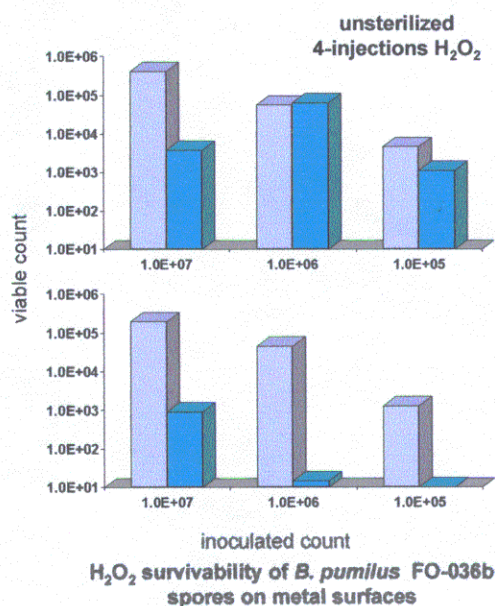


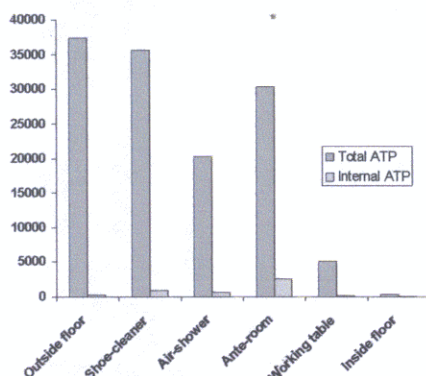
Figure 1. Phylogeny of microbes isolated from JPL's spacecraft assembly facility. Strains resistant to H₂O₂ vapor sterilization are highlighted.

with spacecraft-quality paints for ~7 to 9 months. In the initial studies reported here, we have examined the total cultivable aerobic heterotrophs, and heat-tolerant (80°C for 15-min.) spore-formers. The results showed that the witness plates coated with spacecraft quality paints attracted more dust particles than the non-coated stainless steel witness plates. Among four paints tested, witness plates coated with NS43G



Maintenance Technologies

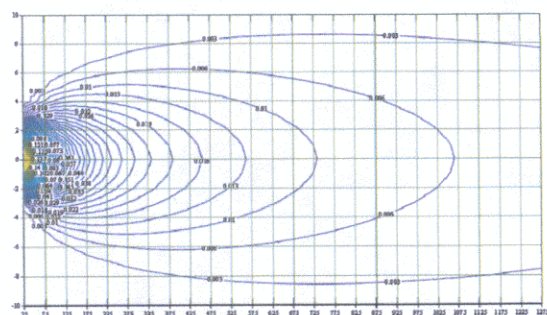
We have conducted a detailed mapping of the biological cleanliness of 14 JPL clean room facilities. The biological cleanliness was measured using an ATPase assay capable of measuring both



intracellular and extracellular ATP present in samples.

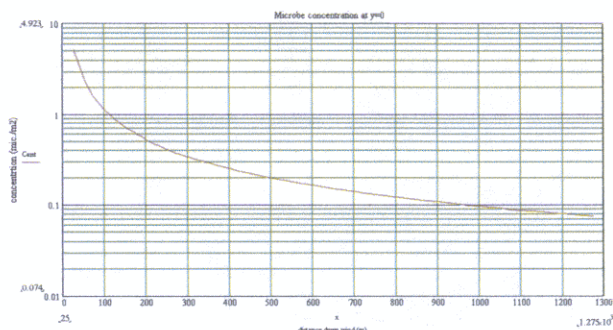
Cross-contamination modeling

To enable reliable in situ, or sample return, life detection missions, it is critical that flight hardware be free of any biological materials that originated from Earth. Therefore, it is important that likely cross-contamination mechanisms be thoroughly studied and understood. Three simple models have been developed to estimate the maximum soil contamination that could originate from a bio-contaminated lander. All three models estimate the ground contamination concentrations at various distances from the lander. The first model estimates the ground concentration if the microorganisms covered the soil 360° around the lander. The second model uses a steady state Gaussian plume to transport the microorganisms from the Lander. The third model determines the ground contamination level from an instantaneous Gaussian puff release, probably at the



time of landing. Input to the models includes the total spacecraft (s/c) contamination level, the height of the lander, the size distribution of the particles, and their microbial burden, the fraction of the total contamination that is removed, the wind speed, and

the diffusivities of the plumes. The results are given for input data available from old studies performed at the Kennedy Space Center. More realistic data are now being obtained at JPL.



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